



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Brow *et al.*
Serial No.: 08/520,946
Filed: 08/30/95
Entitled: **Rapid Detection and Identification
of Pathogens**

Group No.: 1636
Examiner: W. Sandals

**DECLARATION OF MARY ANN D. BROW
UNDER 37 CFR §1.132**

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)	
I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Box Non-Fee Amendment, Assistant Commissioner for Patents, Washington, D.C. 20231.	
Dated: <u>November 2, 1998</u>	By: <u><i>Kamrin T. MacKnight</i></u> Kamrin T. MacKnight

1. I, Mary Ann D. Brow, am one of the joint inventors of the subject matter embodied in the above-identified patent application.
2. I am familiar with the Final Office Action from the Patent Office mailed August 31, 1998 in the above-named application (*i.e.*, U.S. Patent Appln. Ser. No. 08/520,946).
3. In the Final Office Action, the Examiner rejected Claims 1, 3-29 and 31-54 as being allegedly obvious under Lyamichev *et al.*, in view of Young, Seela and Roling, and Young *et al.* I am a co-author of Lyamichev *et al.* and contributed to and am familiar with the experimental data in Lyamichev *et al.*
4. The experimental findings in Lyamichev *et al.* teach away from the use of intra-strand secondary structures as targets for cleavage means. Instead, the experimental findings in Lyamichev *et al.* teach the use of cleavage structures formed by inter-strand annealing between a primer and a target nucleic acid. In the absence of primer, target nucleic acid molecules were cleaved poorly, or not at all. Specifically, as described on page 782 of Lyamichev *et al.*, we showed that cleavage of RNA substrates was dependent on the presence

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of a primer. Additionally, as described on page 780, column 1 of Lyamichev *et al.*, we obtained optimal cleavage of DNA substrate in 50 mM KCl in the presence of a primer. In the absence of primer at this optimal KCl concentration, cleavage was almost completely undetectable (See Figure 3B). When buffer conditions were optimized for cleavage without primer, reactions had to be incubated for approximately fifteen times longer to obtain a similar degree of cleavage as compared to reactions containing primer.

5. The experimental findings in Lyamichev *et al.* also demonstrate that a primer is required to control the location of the cleavage event. For example, as described on page 779 of Lyamichev *et al.*, we showed that, in the absence of primer, cleavage occurred at the ends of the substrate duplexes between the first and second base pairs and that the primer-directed shifting of the site of cleavage suggested that precise orientation of the 5' nuclease on the substrate was dominated by the interaction of the polymerization domain of DNAP-Taq with the primer.

6. Lyamichev *et al.* further teaches that a primer is important for applications using the cleavage reactions. On page 782 of Lyamichev *et al.*, we explain that the use of primers greatly increases cleavage efficiency, and reduces the number of unwanted cleavages at regions of secondary structure in the target nucleic acid. Without primer, we found that the location of the cleavage was unpredictable, undesired cleavage was caused by exogenous DNA (contaminant DNA), and undesired cleavage occurred at regions of secondary structure in the target nucleic acid. Thus, in Lyamichev *et al.*, we taught that primers were needed for applications using the cleavage reactions and that cleavage of intra-strand secondary structures as targets in cleavage reactions was undesired.

7. The undersigned declares further that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: Nov. 1, 1998

Signed: _____

Mary Ann D. Brow

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